

**In the Specification**

Please replace the following paragraphs with the following rewritten paragraphs.

Please insert the following paragraphs beginning on page 7, after line 15:

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 shows the location and number of the CB peptides for the  $\alpha 1$  chain of human type II collagen and the  $\alpha 1$  chain of bovine type II collagen. CB peptides have been numbered using chick collagen as the standard. Arrows represent methionine residues in the human type II sequence and the bovine type II sequence which are the sites of cleavage by cyanogen bromide.

Figure 2a show a carboxymethylcellulose (CM-cellulose) chromatogram and corresponding Coomassie blue protein stain illustrating the separation of the CB peptides of bovine type II collagen, using a linear salt gradient (.....).

Figure 2b shows Coomassie blue stained gels of purified fractions from the CM-cellulose column containing various CB peptides, including CB10 and CB11, of bovine type II collagen.

Figure 3 shows the levels of IgG antibodies to human type II collagen in patients with rheumatoid arthritis (RA), psoriatic arthritis (PsA), and osteoarthritis (OA), and in normal human sera (NHS). Results are expressed as the number of standard deviations (SD) above the mean level of antibody in normal sera. A serum is regarded as giving a positive test result if the level of antibody is greater than 3 SD above the mean (—). The thick dashed line represents the mean level of antibody for each group of patients.

Figure 4 shows the levels of antibodies to the CB10 peptide of bovine type II collagen in patients with rheumatoid arthritis (RA), psoriatic arthritis (PsA), and osteoarthritis (OA), and in normal human sera (NHS). Results are expressed as the number of standard deviation (SD) above the mean level of antibody in normal sera. A serum is regarded as giving a

positive test result if the level of antibody is greater than 3 SD above the mean (—) (---). The thick dashed line represents the mean level of antibody for each group of patients.--

Please delete the paragraphs beginning on page 12 at line 3 and ending on page 13 at line 4.

Please replace the paragraph beginning on page 14 at line 15 with the following, rewritten paragraph:

The CB peptides were initially resolved into 2 major fractions by chromatography on a 2.4\_x\_82 cm column of Bio-Gel P-2 (100-200 mesh, exclusion limit 2600 daltons, globular proteins, Biorad, USA) equilibrated with 0.1 M acetic acid. The peptides obtained after cyanogen bromide digestion were dissolved in 12 ml of 0.1 M acetic acid and 3 ml (~500 mg) was applied to the column at a time. The larger peptides, eluted in the excluded volume of the P-2 column, were pooled, lyophilized, and rechromatographed on a 2.5\_x\_12 cm cation exchange column of carboxymethylcellulose (CM-cellulose, Whatman CM-52). The column was equilibrated with 0.02 M sodium citrate, pH 3.6 containing 0.01 M NaCl, designated as the start buffer, and the peptides were dissolved in this start buffer for application to the column. Chromatography was performed at 42-°C over 10 hours using a linear salt gradient obtained by the addition of 1% 0.16 M NaCl in 0.02 M sodium citrate, pH 3.6, to the start buffer every 6 minutes, with a flow rate of 1.75 ml/mm, using the Biorad Econo system (Biorad, USA). Fractions of ~17 ml were collected at 10 minute intervals, and were run on 15% tricine gels and stained with 0.2% (w/v) Coomassie Blue (Biorad, USA). Fractions containing CB10 identified by comparison with published type II CB peptide maps (Bornstein *et al*, 1980; Miller *et al*, 1982) were pooled, as were fractions containing CB11, desalted on the Bio-Gel P-2 column and lyophilized. CB10 was purified further, and in particular separated from CB8 and CB9,7, and CB11 was separated from CB12, by applying each fraction to a 1.0 x 75 cm column of P-30 (100-200 mesh, exclusion limit 40kD, globular proteins, Biorad, USA) equilibrated in 0.1 M acetic acid. The purity of the peptides after each column run was determined by separation on 15% tricine gels and staining with 0.2% (w/v) Coomassie blue (Fig. 2a, 2b).

Please replace the paragraph beginning on page 15 at line 9 with the following, rewritten paragraph:

Purified CB10 and CB11 were renatured by step-wise cooling from 20°C to 0.2°C, as described by Terato *et al*, 1985.

Please replace the paragraph beginning on page 15 at line 14 with the following, rewritten paragraph:

Antibodies to native human type II collagen, and native bovine type II collagen, were measured by an enzyme linked immunosorbent assay (ELISA). Microtitre plates (Dynatech, Germany) were coated with 100  $\mu$ l/well of 10  $\mu$ g/ml human or bovine type II collagen overnight at 4°C. Plates were washed 3 times in 1% (w/v) skim-milk powder in PBS, pH 7.4 (SM-PBS) containing 0.05% ~~Tween~~ TWEEN 20 (Sigma, USA) and 3 times in distilled water (dH<sub>2</sub>O), blocked with 1% SM-PBS, 0.05% ~~Tween~~ TWEEN 20 for 2 hours at room temperature, then washed again as above. Sera were tested in duplicate at a dilution of 1:50 in the presence or absence of antigen. Antibodies were detected using horseradish peroxidase conjugate (HRP) anti-human IgG (Silenus, Hawthorn, Australia), with 0.5 mg/ml 2,2-azino-di-[3-ethyl-benzthiazoline sulfonate (6)] (ABTS) (Boehringer, Germany) in 0.03 M citric acid, 0.04 M Na<sub>2</sub>HPO<sub>4</sub>, 0.003% H<sub>2</sub>O<sub>2</sub>, pH 4 as substrate. Plates were read after 30 minutes at 415 nm on a Biorad platereader (Biorad, USA). Non-specific binding of immunoglobulins to the plates was allowed for by subtracting the optical density (OD) obtained in the absence of collagen from that obtained in wells coated with collagen.

Please replace the paragraph beginning on page 16 at line 13 with the following, rewritten paragraph:

Antibodies to the purified CB 10 peptide of bovine type II collagen were measured by ELISA as described above for intact type II collagen with the following modifications. Plates were coated with 100  $\mu$ l of 10  $\mu$ g/ml of purified CB10 peptide and sera were tested at a dilution of 1:50. The peptide antigen was coated onto the plates overnight at 4°C, blocked with 1% SM-PBS, 0.05% ~~Tween~~ TWEEN 20 for 90 minutes at room temperature with shaking and sera were incubated for 2 hours at room temperature with shaking. Plates were

washed 3 times in tris buffered saline (TBS) containing 0.05% ~~Tween~~ TWEEN 20 using an automatic plate washer (Wallac, Finland) in all steps prior to the addition of the secondary antibody and in distilled water) immediately before and in all steps subsequent to the addition of the secondary antibody. Bound antibodies were detected using HRP-conjugated anti-human IgG (Silenus, Hawthorn, Australia), and the appropriate substrate. Results are expressed in terms of the number of standard deviations above the mean for controls as described above.